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## Original communication

## Generating STR profile from "Touch DNA"

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#### ABSTRACT

As forensic DNA technology has become a common tool in criminal investigations, scientists have attempted to obtain DNA evidence from what were once considered unlikely sources. "Touch DNA" refers to the DNA that is left behind from skin cells when a person touches or comes into contact with an item. This present study shows, DNA profiling of touched evidence materials is reported employing a combination of LCN typing and miniSTRs. The technology is highly valuable for increasing the scope of DNA profiling to large number touched evidence materials.

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#### 1. Introduction

Humans shed tens of thousands of skin cells each day, and these cells are transferred to every surface our skin contacts. When a crime is committed, if the perpetrator deposits a sufficient number of skin cells on an item at the scene, and that item is collected as possible evidence, touch DNA analysis may be able to link the perpetrator to the crime scene. DNA profiling from swabs of palm and touched items like handles, pens, phones etc was first demonstrated in 1997.<sup>2</sup> Increasingly touched evidence materials useful for crime investigation are subjected to genetic profiling. Touch DNA doesn't require us to see anything, or any blood or semen at all. It only requires seven or eight cells from the outermost layer of our skin. Touch DNA has been successfully sampled from countless items including mobile phones, gun grips, steering wheels, eating utensils, and luggage handles, just to name a few.<sup>3</sup> However, since Touch DNA is usually deposited in smaller amounts than the DNA found in bloodstains or other body fluids, it is more difficult to obtain DNA profiles from touch DNA samples. The key to obtaining successful Touch DNA results depends on recognizing items which may be suitable for Touch DNA analysis and using the sampling technique that will recover the highest number of skin cells.

Despite the potential exchange of material, a forensic scientist may encounter difficulties when trying to recover DNA, particularly if there is extremely low-level DNA and/or damage to the DNA due to degradation or contamination from external factors. In the present study, DNA profile is generated from swabbed latent fingerprint by LCN (low copy number) typing and use of miniSTRs. The contested issue in legal argument over DNA evidence is now less often the identification of the sample, but rather issues such as transfer, persistence, and exactly how that sample came to be present at the crime scene. 4 Often there is not sufficient DNA present on an item for analysis with standard STR typing protocol, the present study shows the importance of developing genetic profile from touched materials has enough and extended repercussions in forensic case evidence collection and analysis for low copy number DNA profiling. This technology is highly valuable for increasing the scope of DNA profiling from touched evidence materials. In an attempt to explore the issues of touch DNA may play an important role as forensic evidence, this study develops the miniSTR protocol for generating the STR profile from highly degraded low template DNA. The technique has dramatically increased the number of items of evidence that can be used for DNA detection.

#### 2. Materials and methods

Cotton swabs (Fig. 1) from touched handles of the spade and slate in a burglary case were received for DNA Profiling along with control blood sample of the suspected burglar for comparison purposes. These touched evidence samples and control DNA samples from suspect were processed separately to avoid the DNA contamination from further generation of false genetic profile. DNA from the cotton

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Fig. 1. DS- Latent fingerprint dry swab from the handle of the spade; WS- Latent fingerprint wet swab from the handle of the small spade; WS1- Latent fingerprint wet swab from the handle of the slate.

swabs were extracted by phenol-chloroform extraction method followed by concentration of aqueous layer to 30 uL by Microcon® 50. DNA was quantitated using Quantifiler® Human DNA Quantification Kit according to the manufacturer's protocol employing 7500 Real-Time PCR System (Applied Biosystems, Foster city, CA). PCR amplification was performed with AmpFISTR® Identifiler® and AmpFISTR® MiniFiler™ PCR Amplification Kits with reduced reaction volume of 12 uL and recommended number of PCR cycles on GeneAmp® PCR System 9700 (Applied Biosystems, Foster City, CA). For LCN typing, 0.5 units of AmpliTaq Gold® (Applied Biosystems) DNA Polymerase were added to the amplified product. With a prehold at 95 °C for 11 min, the products were amplified for 6 additional cycles. Negative controls and reagent blanks were tested to check the contamination and all the amplification reactions were conducted twice to assess consistency. Amplified products of the two STR multiplexes were electrophoresed on ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems) with POP4 polymer and the data was analyzed using GeneMapper® ID Software v3.2 (Applied Biosystems, Foster City, CA).

### 3. Result and discussion

Real-Time PCR could detect approximately 2, 6 and 20 pg/µl of human DNA from three touched evidence swabs. No amplification could be observed from the first two samples while weak amplification for only few loci could be observed from the third sample having about 20 pg/µl DNA. The quality of the genotypic result for miniSTR multiplex (Minifiler) amplification was better than the commonly employed STR multiplex (Identifiler) amplification. The partial profile was generated by AmplSTR® Identifiler™ even after LCN typing with base line noise and unwanted allele peaks, so that the generated profile was not considered further for the genetic profile comparisons. The miniSTR profile further improved by LCN

typing<sup>5</sup> and full profile was obtained from the swab of touched evidence having 20 pg/ $\mu$ l of human DNA. The partial and complete genetic profile obtained from the swab matched with the profile of suspected burglar at all loci as shown in Table 1. Typing of DNA with MiniFiler kit<sup>6</sup> samples as low as optimal DNA quantity of 40 pg/ $\mu$ l or above has also been reported.<sup>7</sup> Partial profiles were obtained from the other two swabs even after LCN typing. Further increase in the number cycles resulted in additional peaks making interpretation difficult. The sensitivity of the DNA profiling method can be improved simply by increasing the number of PCR cycles. The increase in sensitivity opens up the potential of profiling finger-prints and many more touched evidence than previously imagined. The improved sensitivity however leads to additional issues in quality control and profile interpretation (Fig. 2).

Use of negative controls with PCR master mix and replicate testing is must for comparison of data to check the spurious and false allele calls causes by any other contaminants. Majority of the complications observed for LCN DNA profiling are due to the stochastic variations like increased stutter peaks, allele drop-in or drop-out and heterozygote imbalance.<sup>8</sup> Due to the random nature of the stochastic variations, they do not amplify identically in replicate analysis.

This study shows that it is possible to recover DNA from touched evidence materials for positive identification. A LCN and miniSTR based strategy generated satisfactory genotyping from few copies of DNA recovered from the touched objects. Traces of biological materials are often found in many cases and the conventional methodologies sometime fail to provide the desired information from them. The study presents the use of miniSTRs as an alternative tool for typing the low copy number DNA samples. Further development and validation of the miniSTR multiplexes may allow profiling of problematic samples. The benefit of implementing this protocol with increased sensitivity is to expand the scope of DNA

**Table 1** miniSTR profile generated from touched evidences by LCN and compared with control blood sample.

Sample	D13S317	D7S820	D16S539	D18S51	CSF1PO	FGA	D2S1338	D21S11	Amelogenin
Handle of spade	9, 11	10, 11	11, 12	14, 15	10, 12	23.2, 25	16, 18	29, 30	XY
Handle of slate (partial profile)	9, 11	10, 11	11				16, 18	29, 30	XY
Blood sample	9, 11	10, 11	11, 12	14, 15	10, 12	23.2, 25	16, 18	29, 30	XY

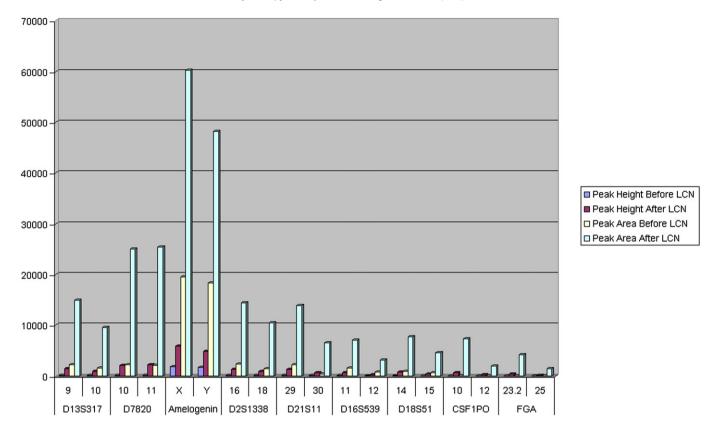


Fig. 2. The graphical representation of STR allele peak height and area difference between pre and post LCN typing of touch DNA sample collected from Spade handle.

profiling to large number of touched materials or degraded samples with traces of DNA. The recovery and handling of touched DNA samples require extreme caution and restriction to avoid secondary transfer of DNA.  $^{10}$ 

A LCN and miniSTR based strategy generated satisfactory genotyping from few copies of DNA recovered from the touched objects. Traces of biological materials are often found in many cases and the conventional methodologies sometime fail to provide the desired information from them. The study presents the use of miniSTRs as an alternative tool for typing the low copy number DNA samples. Further development and validation of the miniSTR multiplexes may allow profiling of problematic samples. The benefit of implementing this protocol with increased sensitivity is to expand the scope of DNA profiling to large number of touched materials or degraded samples with traces of DNA. 11 The recovery and handling of LCN samples require extreme caution and restriction to avoid secondary transfer of DNA. The comparison of duplicate results was used to successfully identify the spurious peaks and provide a consensus profile. It is also essential to develop standard LCN typing protocols from sample recovery to data interpretation.<sup>12</sup>

#### 4. Conclusion

The key to obtaining successful Touch DNA results depends on recognizing items which may be suitable for Touch DNA analysis and using the sampling technique or collection method that will recover the highest number of skin cells. This study shows that it is possible to recover DNA from fingerprints with qualitative and quantitative features suitable for positive identification. This LCN typing protocol with miniSTRs permitted to obtain satisfactory genotyping from few copies of DNA recovered from fingerprints from the spade handle surface. However, if this type of evidence

being affected by the secondary shedding effect, it will be difficult for investigator to interpret the DNA profile as it may generate a mixed profile. The usefulness of this technique will ensure its continued use in forensic investigation further research into DNA transfer and LCN DNA profile interpretation would increase its evidential value and decrease misconception of DNA profiling methodology. Our further studies are being carried out in this direction to resolve such issues for low copy number DNA sample analysis because now the low copy number technique we like to opt for routinely collected touched DNA from suspects, victims and crime scene for examination by this method.

The overall results presented concerning the use of commercial kit AmpFISTR® MiniFiler™ (Applied Biosystems) systems as an alternative method for STR profile generation are indeed encouraging for analyzing the low copy number DNA samples. Further development and validation of these systems may in the near future allow for full STR profiles to be generated from previously problematic materials. The benefit of implementing this protocol with increased sensitivity as well as ability to examine highly fragmented DNA is necessary for future forensic crime case analysis.

Conflict of interest None declared.

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Ethical approval

Approved by institutional ethical committee.

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